ZOOLOGICAL SCIENCE 10: 913–924 (1993)
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Axotomy-induced Long-lasting Firing in an Identified Crayfish Motoneuron

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ABSTRACT—An anal motoneuron L (AML) of *Procambarus clarkii* has its soma in the 6th abdominal ganglion (A6) and its axon extends along the posterior intestinal nerve (PIN) out from A6, but how far its ascending process runs forward through the nerve cord remains unknown. In this study, identified AML activity was recorded from PIN in the isolated nerve cord preparation which composed of thoracic (T1-T5) and abdominal ganglions (A1-A6) with PIN. Axotomy at any level below A1, or at a more distal position, induces repetitive firing in an otherwise silent AML. The firing showed a characteristic discharge pattern and persisted for more than an hour ($\leq$67 min, N=108). The duration and the latency of firing became shorter while the frequency increased as the axotomy was performed more caudally. The response was not blocked by bathing the nerve cord in high Ca^{2+}/Mg^{2+} saline or Ca^{2+}-free saline, but was stopped by ligature of the nerve cord caudal to the axotomized site or by application of a hyperpolarizing pulse to this region. A depolarizing pulse instead enhanced firing. This indicates the axotomy-induced firing originates in AML itself and results from depolarization occurring at the cut end and also an ascending axonal process of AML runs through the nerve cord rostrally up to A1. but not beyond this ganglion.

INTRODUCTION

Effects of axotomy on neuronal excitability (electrogenesis) have been investigated in many vertebrate and invertebrate neurons. A variety of cases of increased, decreased, or unchanged excitability in axotomized neurons have been reported [24]. In invertebrate neurons, axotomy can induce a somatic excitability in which normally non-spiking somata of the crayfish, *Procambarus clarkii* or insect motoneurons and interneurons produce fast rising, overshooting action potentials in response to direct current injection after peripheral axotomy or local application of colchicine [19, 9, 14, 20]. All of these enhanced excitability, which lasted for only a limited time, were observed several hours to a few days after axotomy [14, 20, 24].

On the other hand, the axotomy-induced excitability in peripheral regions of severed invertebrate neurons has not yet been elucidated [24], although several studies have shown that the resting membrane potential shows a decrease at the severed site in the earthworm [13], cockroach [21] and *Aplysia* [25]. In crayfish and lobster, the severed motor and giant axons separated from their somata can survive, conduct action potentials and release transmitter for more than 150 days [1, 3, 17]. However, in *P. clarkii*, Kuwada and Wine [14] did not find any neurons capable of exhibiting a peripheral excitability to axotomy, but found that all phasic neurons tested expressed somatic excitability, while the excitability of spontaneously active or tonic type neurons was unaltered.

I have reported previously in *P. clarkii* that an anal contractor motoneuron has its soma in the terminal abdominal ganglion and the axon extends to the anal musculature out from this ganglion [15, 16]. However, how far its ascending axonal process runs forward through the nerve cord remains to be clarified. In this paper, this identified motoneuron is shown to exhibit peripheral firing following axotomy, and the mechanisms underlying this firing are presented. On the basis of such a unique property of this motoneuron, a possible

Accepted September 20, 1993
Received April 19, 1993

innervation of its ascending process is clarified physiologically.

MATERIALS AND METHODS

Preparation

The crayfish *Procambarus clarkii,* which was obtained from a commercial source, was used throughout the experiments. The entire length of nerve cord from the 1st thoracic (T1) to the 6th abdominal ganglion (A6) was dissected free from the animal. Six pairs of nerves and one unpaired intestinal nerve (IN) originates from A6 (Fig. 1, inset) [16]. IN branches further into 3 branches, the posterior intestinal nerve (PIN) and the pairs of the anterior intestinal nerve (AIN). PIN was left intact attaching to A6, while AINs were trimmed off. The experiments were performed either on this thoracico-abdominal nerve cord preparation or the preparation in which the nerve cord was cut selectively between T1 and A6.

The preparation was placed in a small chamber filled with saline consisting of 208 mM NaCl, 5.4 mM KCl, 13.3 mM $CaCl_2$, 2.6 mM $MgCl_2$, buffered with 10 mM-Tris at pH 7.5, and kept at a low temperature ($<11^{\circ}C$). The chamber consisted of two or three compartments separated by a bank of grease (silicone lubricant) (see Figs. 5, 6, inset). In one compartment, the nerve cord was transected with scissors, ligated with surgical silk, or exposed to the bath application of test solutions. In stimulation experiments, the chamber with three compartments was used, which allowed selective stimulation of either polarizing current supply without disturbing recording of PIN activity. The PIN activity was monitored in the end of compartments.

Identification and recording

The anal motoneuron L (AML), which is capable of driving rhythmic anal contractions, can be identified by its largest amplitude among units recorded from PIN [15, 16]. PIN divides into two equal branches at a distal region [16] and the discharge activity of AML can be recorded extracellularly with a suction electrode from either of the distal branches of PIN (see the diagram in Fig. 1). The AML activity was displayed on a pen recorder *via* an amplifier and stored on magnetic tape. Spike intervals were calculated with a signal processor (7TO7A, San-ei Instr. Co., Japan).

Bath application experiments

The saline in the compartment where the nerve cord was transected was replaced by high-Ca^{2+}/Mg^{2+} saline or Ca^{2+}-free salines. The high-Ca^{2+}/Mg^{2+} saline contained Ca^{2+} and Mg^{2+} 2.5 times of the normal concentration. Ca^{2+}-free salines contain either 15.9 mM MgCl or 1 mM EGTA. In this study, these salines are referred to as high-Mg^{2+} and Ca^{2+}-free saline, respectively. All were buffered with Tris to pH 7.5.

Stimulation experiments

For stimulation or current passing, the chamber with three compartments was used. The first two adjacent compartments were equipped with Ag-AgCl electrodes (S1 and S2 in Fig. 6), through which the stimulation and the polarizing current was passed. Methylene blue vital staining indicated that the ascending process of AML runs rostrally through the nerve cord beyond A5 on the ventral median surface of the cord (unpublished observation). Thus, the partition between these two chambers were usually placed between two ganglia anterior to A5. A battery-operated (1.5–6 V) stimulator with microammeter to monitor the current strength was connected to the electrodes.

Either hyperpolarizing or depolarizing current (pulse) was applied to the various positions of the cord or its transected end through these electrodes, changing the polarity by means of the switch placed in the circuit (Fig. 6, inset).

RESULTS

AML appears to be a T-shaped unipolar cell, because its soma is located in A6 and its axon innervates the anal musculature *via* PIN [15, 16] and its ascending process runs rostrally through the nerve cord beyond A5. Then, the effect of axotomy on AML activity was examined by cutting the nerve cord and the PIN nerve at various levels.

Transection experiments

First, the effect of transection of the thoracic nerve cord was examined in the thoracico-abdominal nerve cord preparations (N=16). Before cutting, AMLs were quiescent in all cases (15/16=94%) except one (1/16=6%) which fired spontaneously at low frequency (≤0.1 Hz). When the connectives between T1 and T5 were transected at any positions, no effect was observed in discharge activity in AML, but transection between T5 and the rostral margin of A1 (e to f in Fig. 1A) normally (13/16=81%) produced firing of AML at a low frequency (Fig. 1A). In this case, a long delay between the transection and the beginning of AML firing was observed (1.4–2.6 sec, mean=1.9 sec, N=5).

Next, the abdominal nerve cord preparations were transected which were composed of A1 to A6 with PIN. Transections at various positions along its long axis between A1 and A6, always caused a sudden increase in frequency of AML discharge

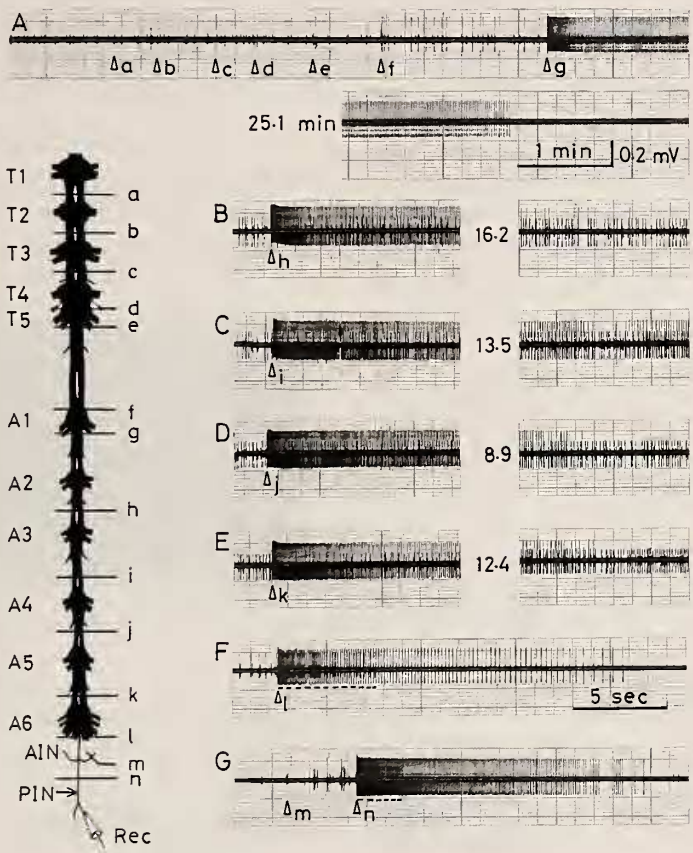

FIG. 1. Response of AML to transection (axotomy) at various positions in the isolated nerve cord preparation. The diagram on left-hand side shows transection positions. The preparation was cut at triangles with the letters, a-n, which correspond to the positions of lines with a-n in the diagram. Note that axotomy at any level below A1 (at g) could always induce LLF in AML (largest spikes), while that above the rostral margin of A1 (at f) could not. A-E, response to axotomy of the nerve cord. The initial and the last parts of a LLF are shown and the figures indicate the time lapse (min) from the start of a LLF. F, response to axotomy of the nerve containing the anterior intestinal nerve (AIN) and the posterior intestinal nerve (PIN). G, response to axotomy of the distal stump of the AIN (at m) and of the proximal stump of PIN (at n). Other neurons (small spikes) contained in PIN responded to axotomy of the nerve (F, G; dotted lines). T1-T5, the 1st to 5th thoracic ganglion; A1-A6, the 1st to 6th abdominal ganglion; Rec, recording position. A, B-E and F, G are different preparations. Time scale was the same which is shown in A, except F.

(Fig. 1A to E), which persisted a while with a characteristic pattern of firing (Figs. 2, 3). This long-lasting firing (LLF) was also observed by recutting the nerve cord stump. In this case, when PIN remained connected to the anal region, the response to transection could actually produce anal contractions as described previously ([16]; Fig. 2F).

This response was also observed by cutting the preparation between A6 and the nerve, containing PIN and even by recutting of the nerve completely isolated from A6 (at n in Fig. 1G), in which there is AML axon but no soma. It seems that the AML soma located in A6 [15] is not necessary for the generation of the axotomy-induced LLFs.

Other neurons contained in PIN did not exhibit such a response. As shown in Figure 1A-E, PIN never responded to transections of the nerve cord at any level. Though transections of the nerve (at l and n in Fig. 1F, G) sometimes (5/17=29%) elicited a low-frequency firing, this firing never persisted like as that of AML (86 msec to 33 sec, mean±SE=11.7±6.1 sec, N=5/17).

In the crayfish, 3 pairs of nerve roots originate from each abdominal ganglion from A1 to A5, and 6 pairs from A6 (Fig. 1, inset) [11, 16]. Cutting of the stumps of any of these side roots failed to evoke discharge activity in AML. Although AIN runs in IN together with PIN, cutting of its distal stump failed to induce any excitability in AML (m in Fig. 1G).

It was then proved that axotomy of the nerve cord preparations along its long axis at any level below A1 can always evoke a prolonged excitability, a LLF of AML.

Discharge pattern of an axotomy-induced LLF

LLF has a characteristic firing pattern. The interspike intervals during LLF were found to increase exponentially at first and reach a steady

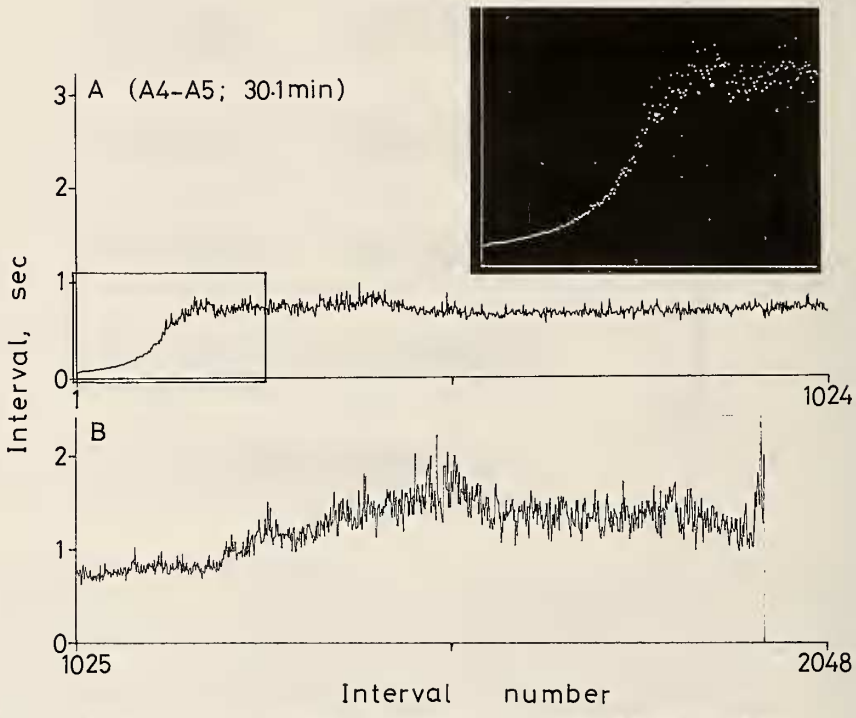

FIG. 2. The plot of sequential spike intervals against interval number during an axotomy-induced LLF in AML. The LLF began at time 0 when the connectives were cut between A4 and A5, which persisted for 30.1 min. The intervals were calculated with a signal processor; clock, 1 msec. Upper right inset; raster display, enlarged view (×4) of a part enclosed by a square in A. Large dots show at the time of 30 sec and 1 min from the beginning of the LLF. Note that intervals reach almost constant level within 1 min.

state (regular interval) within 30 sec to 1 min, which was maintained over the successive time course with some progressive reduction (Fig. 2). This was common to all cases of transections of different positions along the long axis of the preparations (N=15).

The changes in firing frequency during LLF were calculated by counting the number of AML spikes every per minute over a period of 25 min (Fig. 3). A similar yet somewhat different time course was found depending on the position of the transections. LLF was thus characterized by an initial high-frequency firing with exponential increase in inter-spike intervals during the first minute, followed by a progressively lower frequency over the successive time course (Fig. 3).

Effect of transection level on AML discharge activity

The latency, duration or frequency of the LLFs were found to be affected by the level of transection. The frequency was defined as the number of AML spikes during the first minute of LLF.

They are summarized in Table 1. Although it was often difficult to measure the latency accurately because of its short duration (<10 msec, especially in the case of transections of the nerve segment isolated from A6), the latency tended to decrease as the preparation was cut more caudal. The duration was found to be variable with the positions of transections in the range of 7 sec to 67 min (N=108, in Table 1). Table 1 shows that, as the transection was done more caudally, the duration became shorter. The frequency, on the other hand, tended to be higher with more caudal transection, ranging from 1.2 to 12.2 Hz (N=101, in Table 1).

In summary, AML begins to fire more rapidly and exhibits more stronger excitability, but its activity stops sooner as the preparation is transected more caudally. These results support the idea that the axotomy-induced LLF is a result of

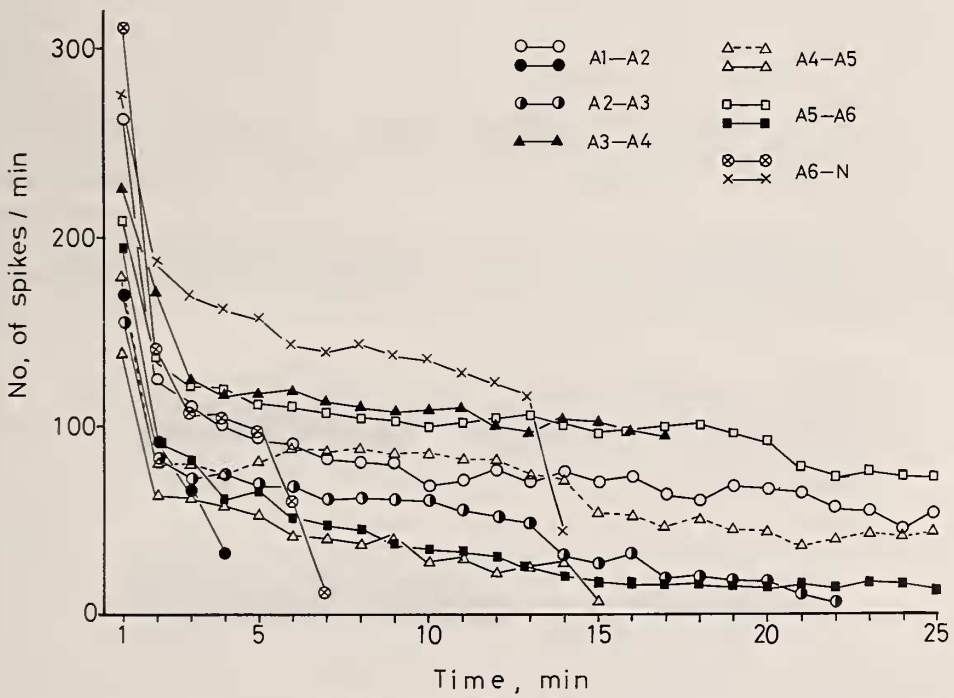

FIG. 3. Time course of the changes in firing frequency during an axotomy-induced LLF in AML. Data from 10 preparations transected at various positions are plotted as the number of AML spikes at 1-min intervals during a period of 25 min after the beginning of a LLF. The LLFs ranged from 4 min to over 25 min. Note the high-frequency firing during the first minute of the LLFs, followed by the lower-frequency over the successive time course. See the diagram in Fig. 1 as for abbreviation for the positions of transection shown at upper right.

TABLE 1. Effect of transection positions on latency, duration and frequency of the axotomy-induced LLFs in AML. The frequency was expressed as the number of spikes during the first minute of a LLF (see Text). The connectives between each two abdominal ganglions (A1 to A6) or the nerve (N) containing PIN were transected

Position of transection	Latency msec	Duration min	Frequency Hz
A1-A2	60± 8.7 (4)	25.3±4.17 (17)	3.3±0.19 (17)
A2-A3	57±22.2 (4)	13.2±2.28 (14)	2.6±0.17 (14)
A3-A4	40± 9.0 (4)	8.4±1.60 (15)	2.5±0.19 (14)
A4-A5	46±13.4 (4)	7.5±2.02 (15)	3.1±0.20 (15)
A5-A6	24± 1.9 (4)	5.6±2.10 (15)	3.6±0.49 (15)
A6-N	16± 1.5 (3)	3.5±1.32 (17)	5.3±0.52 (16)
N-N	<10 (3)	2.8±1.01 (15)	7.4±0.79 (10)

Values are mean±SE (number of preparations).

the axotomy of AML itself and that it initiates at the point of transection.

Bath application experiments

To see whether the LLFs are independent of input from other neurons, the abdominal nerve cord was bathed in high-Ca^{2+}/Mg^{2+} saline bath (N=4) to block interneuronal (polysynaptic) pathways within the ganglions [8], and in high-Mg^{2+} saline (N=6) or Ca^{2+}-free saline (N=2) to block synaptic input [2]. These baths did not inhibit the response of AML to axotomy even after a 2-hr period of adaptation.

The response to axotomy in high-Ca^{2+}/Mg^{2+} saline was similar to that in normal saline, but the response in high-Mg^{2+} or Ca^{2+}-free saline differed from that in normal saline in the prolonged and even reinitiated firing in some cases. Its firing rate was increased and firing pattern was changed (Fig. 4). The AML activity following axotomy in high-Mg^{2+} saline was sustained for a long-period of time ($\geq$1.5 hr in 3/6 cases), up to about 15 hr. In Ca^{2+}-free saline, it persisted for 1.5–3 hr, and the firing pattern changed from tonic to phasic, then later reverted. The change in the firing pattern was observed in 2 cases out of 6 in high-Mg^{2+} saline, and in 2 cases out of 2 in Ca^{2+}-free saline.

In 2 cases out of 6, AML fired spontaneously in a tonic firing pattern 1.5 hr or 4 hr after adaptation in high-Mg^{2+} saline; the high-frequency firing was established gradually (Fig. 4C, D) and persisted for hours (4 hr or 12.3 hr, respectively). There-

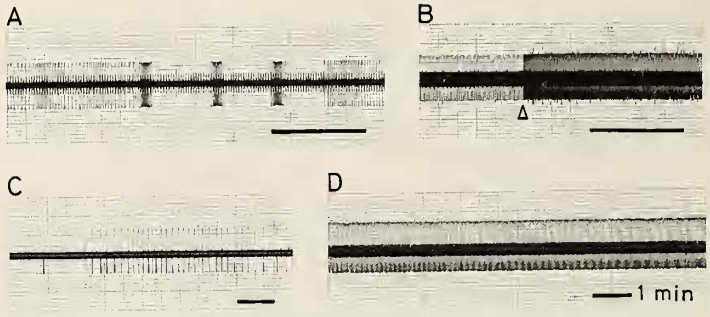

FIG. 4. AML activity in the bath application of high-Mg^{2+} saline ($CaCl_2$ replaced by $MgCl_2$). A, modulation of the firing pattern of AML activity (largest spikes) at 1 hr after application, in which axotomy was performed 30 min after application. B, 130 min after application in the case of A, AML again restarts a high frequency firing following axotomy at △ (between A3-A4). C, after about 4 hr of the adaptation, AML began to fire spontaneously with a gradual increase in firing rate, followed by a high-frequency firing, which persisted for a long period of time. D, about 6 hr after C. A, B and C, D are different preparations.

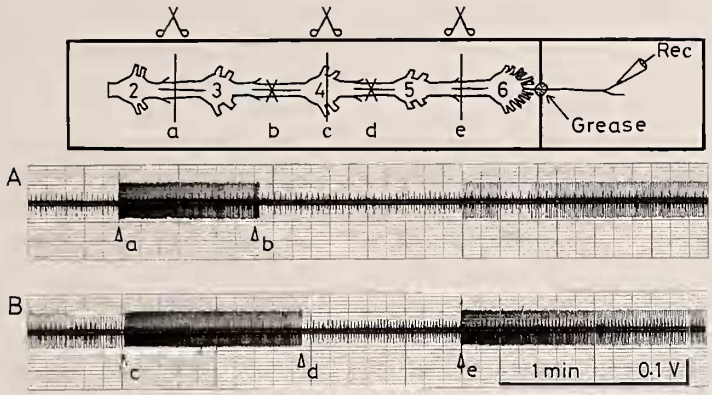

FIG. 5. Blockage of an axotomy-induced LLF in AML by ligature. The LLFs induced by axotomy of the isolated nerve cord preparation (upper inset) at the positions △ with a, c, and e, stop by ligature at the positions △ with b and d, caudal to the axotomized positions. In this case, when the thread used for ligature became loose, AML began to fire at the right side of A. The positions of axotomy (vertical lines) and ligature (×) and the recording position of AML activity (Rec) are represented in upper inset. B is a record about 1 min after the end of A.

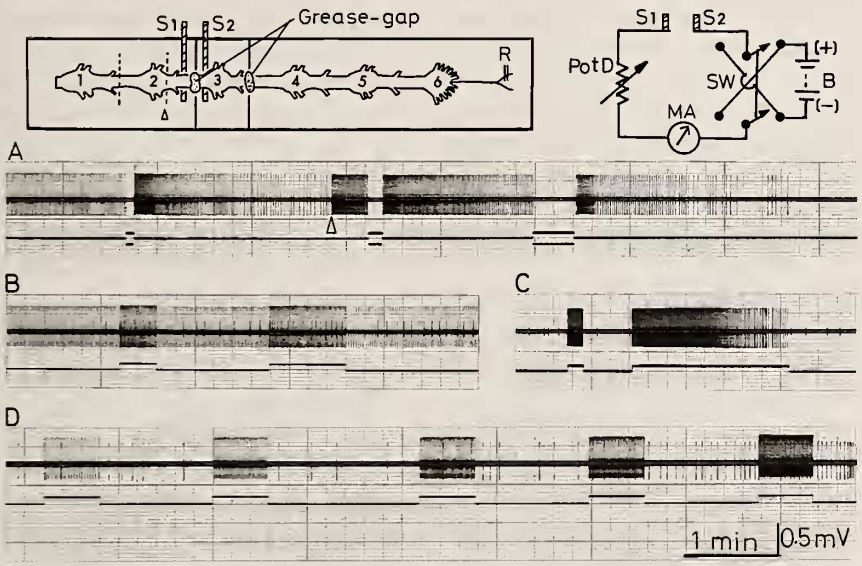

FIG. 6. Response of AML to hyperpolarizing or depolarizing pulse. Upper inset, diagram of arrangement (right) and circuit (left) used for applying stimulation. S1 and S2, stimulation electrodes; R, recording electrode; Pot D, potential divider; MA, microammeter; SW, switch for changing a polarity of S1 and S2; B, battery. A, suppression of the axotomy-induced LLFs in AML by applying a hyperpolarizing pulse to the nerve cord caudal to the position of transection. The LLFs were observed by transection of the nerve cord at first at the position represented as a dotted line and then after at the position with △ in upper inset. B, increase in the frequency of AML activity by applying a depolarizing pulse. C, a prolonged firing in quiescent AML induced by a depolarizing pulse. D, changes in the firing rate of AML with an increase in the intensity of depolarizing pulses (from left, 22, 45, 90, 245, and 399 μA). A, B, C, and D are different preparations. Upper traces represent discharge activities (AML spikes are the largest) and lower traces stimulation; the double horizontal bar indicates the duration of a hyperpolarizing pulse (S1 is negative in respect to S2) and the single bar (upward) that of a depolarizing one (S1 is positive with respect to S2).

fore, the synapse-blocking saline seems to raise the firing ability of AML.

Ligature and stimulation experiments

When the connectives caudal to a transection position were tied with a thread during a LLF, the response always stopped immediately (Fig. 5). If the thread became loose, AML soon resumed its firing activity (Fig. 5A). This fact shows that the LLF might be reversibly blocked by local ligature of the connectives and that the axotomy-induced LLF might originate from the transection site, i.e., the cut end of the AML axon. This possibility was confirmed by the following stimulation experiments.

First, as shown in the inset of Figure 6, a counter current was applied to the nerve cord caudal to the transection position by the two electrodes, S1 and S2, in which S1 in the rostral position close to the cut region is negative, in respect to S2 in the caudal position. This stimulus (hyperpolarizing pulse) was clearly capable of blocking the LLF induced by transection of the nerve cord rostral to the electrodes (Fig. 6A). In this case, a complete blocking of the LLF could be seen if the applied pulse was large enough ($\geq 30\,\mu$A). These facts show that a prolonged excitability of AML seen following transection was produced at the cut end of the AML itself.

Secondly, reversing the polarity of the two electrodes was caused by a depolarizing pulse applied at the cut end. A depolarizing pulse applied during LLF could raise its firing rate (Fig. 6B). Such a stimulus at any position along the abdominal nerve cord could produce a tonic firing in quiescent AML (Fig. 6C). As shown in Figure 6C, the response lasted throughout the stimulation: AML showed a high-frequency firing at the beginning of the stimulation and then a progressive decline. Increase in stimulus intensity also raised the firing rate (Fig. 6D). The response characteristic of the stimulus-induced firing in AML was similar to that of the axotomy-induced firing (compare Fig. 6C with Fig. 1).

The mean latency between onset of stimulus and the start of LLF with standard error was found to be 48 ± 2.0 msec (N=9, in the range of 34–54 msec) when depolarizing pulse was applied at the connectives between A1 and A2. Comparison of this result with that for axotomy-induced response

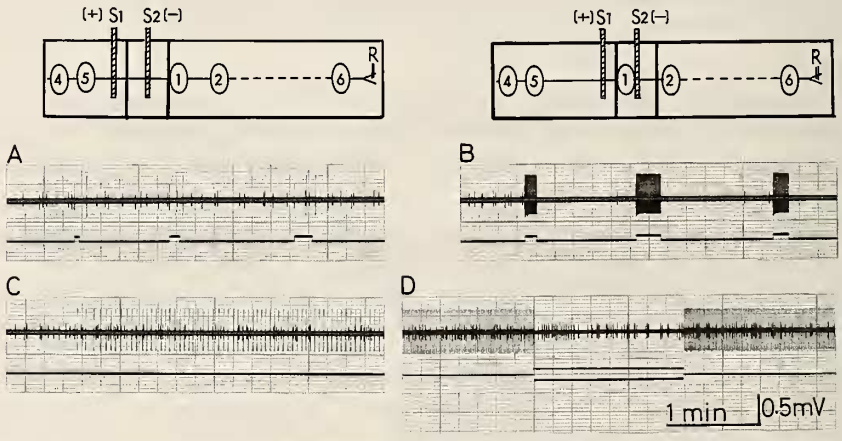

FIG. 7. Illustration of the innervation of an ascending process of AML, reaching up to A1. The arrangements of the preparation composed of T4 (4) to A6 (6) with PIN, the stimulation electrodes (S1, S2) and the recording electrode (R) are shown in upper inset. A depolarizing pulse (120 μA, upward horizontal bar in lower trace) applied above A1, between T5 and A1 (left inset) can not induce LLF in AML (A), while this pulse applied below A1, between A1 and A2 (right inset) can always induce LLF during the pulse (B). C, 9.5 min after B, AML begins to fire spontaneously. D, 75 min after the beginning of spontaneous firing of C, the high-frequency firing of AML is suppressed by a hyperpolarizing pulse (30 μA, double horizontal bars in lower trace) applied by the same electrodes that was used for applying pulse in B (upper right inset), but the polarity of the electrodes was reversed, S1 was negative relative to S2.

(60 ± 8.7 msec, N=4 in Table 1) shows no significant difference ($P<0.05$, using Mann-Whitney U-test).

How far does AML extend its ascending process?

By use of the same procedure described above, direct confirmation of the hypothesis that an ascending branch of AML runs through the nerve cord to A1 but not above it was provided; a depolarizing pulse to the connectives between A1 and A2 produced prolonged discharge activity in AML while the same stimulus to those between T5 and A1 failed to do so (Fig. 7A). This response was similar to that observed by transection of the nerve cord between T5 and A1 (Fig. 1A).

In this experiment AML sometimes began to fire spontaneously a few minutes after cessation of a stimulus-induced LLF. The discharge frequency increased gradually (Fig. 7C, D) and lasted for more than 1 hr. Even after 75 min, this spontaneous LLF was found to be reversibly blocked by a hyperpolarizing pulse applied by the same electrodes as those used for expression of the stimulus-induced LLF (Fig. 7D).

DISCUSSION

Endogenous property of the axotomy-induced LLF

The observation reported here showed that long-lasting firing (LLF) in an identified motoneuron, AML of the crayfish occurs by axotomy of the nerve cord preparation at any level below A1 along its long axis (Fig. 1). Ultrastructural [22] and physiological analysis [11] showed that there are many thousands of axons in the connectives of crayfish nerve cord. There are also sensory cells within the nerve cord of the crayfish, which respond to stretch of the nerve cord [10, 11].

Accordingly, one might expect that the axotomy-induced LLF would be a result of synaptic interactions between AML and other neurons or sensory elements. This possibility is disproved by the bath application experiments with synapse-blocking salines; high Ca^{2+}/Mg^{2+} and Ca^{2+}-free salines did not block the LLFs, but instead Ca^{2+}-free salines increased the firing rate and prolonged the duration. A spontaneous discharge of AML occurred and persisted for a long period of time (>3 hr) in this saline (Fig. 4D). Both initiation and maintenance of the LLFs are possible in synapse-blocking salines, supporting the idea that the LLFs must be due to an endogenous property of AML itself. Invariant characteristics in firing patterns (Figs. 1, 3) and the short latency (Table 1) also support this idea.

It is interesting to note that decay of firing frequency (Fig. 3) and the long duration (7 sec to 67 min, N=108) of LLFs closely resemble those described for a long-lasting afterdischarge of the bag-cells in *Aplysia,* which is induced by a brief train of electrical stimuli [5] or occurs *in vivo* before normal egg laying [18]. In *Aplysia,* the afterdischarge exhibits high frequency firing during approximately 1 min of activity, followed by a low-frequency one and its duration ranges from <1 min to 70 min (N=21) [5]. These similarities in activities of AML and the bag-cells may be due to an endogenous property based on prolonged depolarization.

The prolonged firing following axotomy seems to be unique to AML in PIN (Fig. 1). Other neurons contained in PIN did not respond to axotomy of the nerve cord (Fig. 1A-E). Even if PIN was cut, the response occurred occasionally (5/17=29%) and did not last long (≤33 sec, Fig. 1F, G). AIN contained both afferent and efferent neurons innervating the hindgut of the crayfish and appeared to have synaptic connection with AML because the AML firing pattern was affected by the presence or absence of input from AIN [16]. However, cutting of the distal stump of AIN did not induce LLF in AML (Fig. 1G). This fact supports the idea that only AML is capable of responding to axotomy, though its functional significance is obscure.

AML response with LLF to axotomy appears to be an exception and can not be generalized to other invertebrate neurons. Effects of axotomy on neuronal excitability have been investigated in motoneurons and interneurons of crayfish [14], locust [9], cockroach [19], and cricket [20], yet none of them have shown a prolonged firing following axotomy (for review, [24]).

Mechanisms underlying the axotomy-induced LLF

The site of spike generation in the axotomy-induced LLF seems not to be restricted to a localized region of AML, unlike other ordinary motoneurons in which the initiation of spike is limited at the so-called initial segment. The LLFs arise from any cut region of the axotomized AML axon, indicating peripheral spike generation. Evidence for this is as follows: (1) Axotomy of the nerve cord and the isolated nerve segment separated from A6 always induced the LLFs in otherwise silent AML (Figs. 1, 5); (2) recutting the nerve cord stump or the isolated nerve segment could again restore the LLFs (Figs. 1, 5); (3) as the axotomy was performed more caudally, the latency became shorter whereas the firing rate increased (Table 1). As cutting nerve axons disrupts their ability to conduct action potentials [13] and the soma of AML locates within A6 [15], these facts support the idea that the LLFs build up at the cut end regardless of the presence of the soma.

If the above idea is true, the propagation of excitability through the axon leading to one LLF must be blocked by some experimental procedures applied locally, and this turned out to be the case. The LLFs were proved to be reversibly blocked by local ligature of the connectives (Fig. 5) or by an adequate hyperpolarizing pulse ($\geq 30\,\mu$A), applied caudally to the axotomized region (Fig. 6A). And even after more than 1 hr of the beginning of the LLF, there is complete blockage of propagation by the hyperpolarizing pulse (Fig. 7D). This means that the LLFs might be retained for a long time at the cut end.

It is then concluded that initiation and maintenance of the LLFs are apparently a local event occurring at the cut of the AML axon, supporting the idea that the LLFs result from a peripheral spike generation; however, no such cases have been reported. For crayfish or insect motoneurons and interneurons, several authors found that axotomy induces somatic, but not peripheral excitability [9, 14, 19, 20].

Several lines of evidence support the idea that the LLFs must be based on a prolonged depolarization occurring locally at the cut site. First, they occurred in response to transection of the preparation, but not to ligature, indicating that they may result from an increased inward current (depolarization) through the cut end of the transected AML. Second, a depolarizing pulse generated a repetitive firing in AML throughout stimulation. This response is similar to that observed in the axotomy-induced firing (Figs. 1, 6C). The intensity of depolarization can be positively correlated with the firing rate (Fig. 6D). Third, a hyperpolarizing pulse can suppress the LLFs (Figs. 6A, 7D).

Similar examples are found in tonic stretch receptor cells of the crayfish and lobster [7, 23] and some molluscan cells [5, 12]. In these cases, a prolonged depolarization induces repetitive discharges in a nerve cell whose firing rate is directly related to the magnitude of depolarization [5, 7, 12, 23]. The firing pattern closely resembles that observed in the present AML [13, 23].

It is then expected that a depolarization observed in the cut end of AML might be associated with injury potentials which occur locally at the injured site of a nerve [24]. However, even though injury currents entering through the injured (cut) site of the nerve are found in the transected lamprey nerve cord [4], *Aplysia* metacerebral neuron [21], and cockroach giant neuron [25], they do not develop repetitive firing at the injured site of a given nerve. In contrast, injury currents of AML always elicited a prolonged firing.

It can therefore be presumed that AML has the specific membrane property capable of eliciting a repetitive firing due to injury currents. Investigations of this property will be the subject of further studies.

Architecture of AML

As discussed above, this study has revealed that the innervation of AML can be identified physiologically. On the basis of this finding and the previous cobalt diffusion experiments [15], an architecture of a single unit, AML is assumed to be T-typed unipolar: One process ascends the nerve cord to end in A1 and the other process runs to the anal musculature *via* PIN. The presence of AML axon in PIN has previously been reported [16] where the axotomy-induced LLFs could actually produce anal contractions, as long as PIN re-

mained in contact with the anal region.

The evidence for the view that the ascending axon (interganglionic axonal process) reaches to and ends in A1 comes from the observation that axotomy of the nerve cord along its long axis at any level below A1 induced LLF in AML, while that above the rostral end of A1 failed to produce this response (Fig. 1). Another observation which supports this view is that a depolarizing pulse applied to the connectives between T5 and A1 failed to induce LLF in AML, while the pulse applied to the connectives caudal to A1 did (Fig 7). Even though the hindgut efferent neurons of the crayfish, *Orconectes limosus,* seem to form synaptic contacts with interganglionic processes in the neuropil of A6 [6], AML is unlikely to have such synaptic contact because of the short delay of the LLFs (Table 1) and because no influence of synapse-blocking salines was observed on the generation of the LLFs (Fig. 4). It appears that the ascending axon runs uninterruptedly through the nerve cord until A1 on the ventral median surface and has no side branching because AML never responded to axotomy of any side roots deriving from A1 to A6.

It is then concluded physiologically that AML has a long ascending interganglionic axon, reaching up to A1. The function and morphological details of this ascending part of AML axon remain to be studied.

ACKNOWLEDGMENTS

The author wishes to express her sincere thanks to Dr. Mituhiko Hisada for his useful suggestions.

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